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ABOUT THE JOURNAL

Aims and Scope

Asian Archives of Pathology (AAP) is an open access, peer-reviewed journal. The journal was first published in 2002 under the Thai name "วารสารราชวิทยาลัยพยาธิแพทย์แห่งประเทศไทย" and English name "Journal of the Royal College of Pathologists of Thailand". The journal is a publication for workers in all disciplines of pathology and forensic medicine. In the first 3 years (volumes), the journal was published every 4 months. Until 2005, the journal has changed its name to be "Asian Archives of Pathology: The Official Journal of the Royal College of Pathologists of Thailand", published quarterly to expand the collaboration among people in the fields of pathology and forensic medicine in the Asia-Pacific regions and the Western countries.

The full articles of the journal are appeared in either Thai or English. However, the abstracts of all Thai articles are published in both Thai and English languages. The journal features letters to the editor, original articles, review articles, case reports, case illustrations, and technical notes. Diagnostic and research areas covered consist of (1) **Anatomical Pathology** (including cellular pathology, cytopathology, haematopathology, histopathology, immunopathology, and surgical pathology); (2) **Clinical Pathology (Laboratory Medicine)** [including blood banking and transfusion medicine, clinical chemistry (chemical pathology or clinical biochemistry), clinical immunology, clinical microbiology, clinical toxicology, cytogenetics, parasitology, and point-of-care testing]; (3) **Forensic Medicine (Legal Medicine or Medical Jurisprudence)** (including forensic science and forensic pathology); (4) **Molecular Medicine** (including molecular genetics, molecular oncology, and molecular pathology); (5) **Pathobiology**; and (6) **Pathophysiology**.

All issues of our journal have been printed in hard copy since the beginning. Around the late 2014, we developed our website (www.asianarchpath.com) in order to increase our visibility. We would like to acknowledge that our journal has been sponsored by the Royal College of Pathologists of Thailand. We have the policy to disseminate the verified scientific knowledge to the public on a non-profit basis. Hence, we have not charged the authors whose manuscripts have been submitted or accepted for publication in our journal.

On the other hand, if any authors request a printed copy of the journal issue containing the articles, each of the copied journals costs 450 bahts for Thai authors and 30 United States dollars (USD) for international authors.

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MANUSCRIPT REVIEWERS

CONTENTS

ORIGINAL ARTICLE

UTERINE SARCOMAS AND CARCINOSARCOMAS IN SRINAGARIND HOSPITAL: A CLINICOPATHOLOGICAL CORRELATION

Tritraporn Sawantranon[1], Waritta Kunprom[1], Amornrat Temtanakitpaisan[2] and Pilaiwan Kleebkaow[2*]

1 *Department of pathology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand*

2 *Department of Obstetrics and Gynecology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand*

* Correspondence to: Pilaiwan Kleebkaow, MD. Department of Obstetrics and Gynecology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand. Email: kpilai@kku.ac.th

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Abstract

Background: Uterine sarcomas and carcinosarcomas are aggressive tumors accounting for 3-7%, and 5% of all uterine malignancies with generally poor outcome.

Objective: To describe subtypes, clinicopathological characteristics, and survival rates of uterine sarcomas and carcinosarcomas

Materials and methods: Medical records and histological results of uterine sarcomas and carcinosarcomas between 2010 and 2019 at Srinagarind Hospital, Khon Kaen University were reviewed

Results: 56 cases have been reviewed. Eighteen cases (32.1%) were carcinosarcomas and 38 cases (67.9%) were uterine sarcomas. With exclusion of carcinosarcomas, the first three most common uterine sarcomas were leiomyosarcomas (50%), Endometrial stromal sarcomas (18.4%), and undifferentiated uterine sarcoma (UUS) (13.2%). Most cases were in stage I (33 cases, 58.9%). The 5-year overall survival (OS) and 5-year disease-free survival (DFS) were 40%

with median of 40 months and 34% with median of 15 months. For carcinosarcomas, the 5-year OS was 54% with a median of 60 months and 5-year DFS was 49% with a median of 35 months.

Discussion and conclusions: Carcinosarcomas are better survival than uterine sarcomas. FIGO staging have an impact on DFS, but not on OS. Adjuvant therapy had impacts on both OS and DFS, particularly in late stages of the disease.

Keywords: uterine sarcoma, carcinosarcoma, clinicopathological subtypes, survival rates

Introduction

Uterine sarcomas and carcinosarcomas are aggressive malignancies, which are problematic for diagnosis and management in the Srinagarind Hospital practices. Uterine Sarcomas account for 1% of gynecologic malignancy, and 3-7% of all uterine malignancies [1-2]. They are heterogeneous tumors with several histologic types. According to World Health Organization (WHO) classification of tumors of female reproductive organs, 5th ed. 2020, uterine sarcoma can be classified into two groups [3-4], malignant mesenchymal tumors and mixed epithelial-mesenchymal tumors (adenosarcomas), which originates from Mullerian mesenchymal cells [3-4,6]. In malignant mesenchymal tumors, the two most common histologic subtypes are leiomyosarcomas, and endometrial stromal sarcomas (ESS)[1–5]. The remaining subtypes, including undifferentiated uterine

sarcoma, and other mesenchymal sarcomas are less frequent [5]. Tumor, node, metastases (TMN) and the International Federation of Gynecology and Obstetrics (FIGO) classification of uterine sarcoma is used for staging malignant mesenchymal tumors [6]. For the adenosarcomas, TMN and FIGO staging are the same as uterine sarcomas, but there are some differences in details [6].

Carcinosarcomas account for 5% of all uterine malignancies [4,7-8]. This is a mixed pathological lesion, which show as malignant in both epithelial and mesenchymal components. In the past, it has been classified as a malignant mixed epithelial-mesenchymal tumor and treated as uterine sarcomas. Nowadays it is believed that carcinosarcomas are one of the endometrial carcinomas that have undergone epithelial-mesenchymal transition to exhibit both components. Therefore, carcinosarcomas are classified and staged, according to WHO classification, as high-grade endometrial carcinoma [6,8]. This new classification provides a gap of knowledge with risk factor, prognosis and management. Carcinosarcomas are still analyzed as uterine sarcomas [9]. With the sarcomatous components, carcinosarcomas are still difficult to distinguish from uterine sarcomas, especially in biopsy specimen, and are still problematic in diagnosis in our institutes.

Clinical presentations of uterine sarcomas are nonspecific. Patients may present with vaginal bleeding, a palpable mass, or abdominal pain. Radiological studies cannot distinguished between uterine sarcoma from its benign counterpart [10]. Major risk factors for uterine sarcoma are pelvic radiation, tamoxifen or an estrogen receptor agonist used, and some congenital conditions [4,10-11], while the risk factors for carcinosarcomas are the same as endometrial carcinoma. Some studies reveal that obesity, and a history of diabetes are also associated with uterine sarcomas [12].

The only prognostic factor affecting survival, currently known today, is tumor staging. Treatment modalities are difference due to tumor heterogeneity. Surgery is the standard treatment for all histologic subtypes [2,13-14]. Total abdominal hysterectomy with bilateral salpingo-oophorectomy (TAH with BSO) and cytoreduction are recommended while fertility-sparing procedures or tumor removal is not suggested and should be considered only in specific patient conditions. For adjuvant therapy, radiation is helpful in local regional control of the disease but does not affect on survival. Chemotherapy is not recommended since there is no proven benefit in uterine sarcomas. In contrast to uterine sarcomas, carcinosarcomas are now classified and treated as high-grade endometrial carcinoma, with proven benefits in multimodality treatment (surgery, radiation, and chemotherapy) [15]. Chemotherapy seems to improve overall survival in carcinosarcomas whereas radiation is not.

So, in this study, it was purposed to review uterine sarcomas, including carcinosarcomas, for the past 10 years in Srinagarind Hospital, in order to study clinical and pathological characteristics, treatment, overall survival (OS) and disease-free survival (DFS) and compare this experience to previously published data, to provide clinical data and the treatment modalities for Srinagarind Hospital in the future.

Materials and Methods

This study was a retrospective observational study performed in Departments of Pathology and Obstetrics and Gynecology, Khon Kaen University. All patients diagnosed and registered as uterine sarcoma and carcinosarcoma in the database of the Department of pathology between January 2010 to December 2019 were included. Patients were excluded if clinical data were missing or incomplete. Clinical data, including age at diagnosis, BMI, menopausal status, presenting symptoms, surgical characteristics, histologic types, treatment, date of first relapse and date of death or last follow up, were collected. Recurrence was defined as the appearance of disease after complete treatment (surgery and adjuvant therapy). OS was defined as the period between the diagnosis and the time of death or last followed up. DFS was defined as the period between the time of the first surveillance and appearance of recurrence or last followed up. Progression was defined as metastasis or patient death during treatment. Sections and slides performed with H&E stains and immunohistochemical stains were reviewed by one gynecologic pathologist. Classification and staging were performed according to FIGO and WHO classification systems of tumors of female reproductive organs, 5th ed. 2020. This study was reviewed and approved by the Khon Kaen University Ethics Committee for Human Research based on the Declaration of Helsinki and the ICH Good Clinical Practice Guidelines (HE641060).

Statistical analysis was performed using IBMR SPSSR Statistics software version 26. Frequencies and percentages were used for categorical data. Means and standard deviations were performed in continuous variables, with Student's t-test used for their differences. Survival analyses, including overall survival and disease-free survival, were calculated and compared using the Kaplan-Meier method and log-rank test. Univariate and multivariate analyses were performed using the Cox regression method. All p-values were two-tailed, and a p-value of <0.05 value was considered significant in all analyses.

Results

The initial cases of 61 patients were reviewed. Five cases were excluded since one case was reviewed and diagnosed as endometrioid carcinoma and the other 4 cases were missing data. The remaining 56 patients were 18 cases (32.1%) of carcinosarcomas and 38 cases (67.9%) of uterine sarcomas. The demographic and clinicopathological characteristics are shown in Tables 1 and 2. When compared, the histologic subtypes within uterine sarcoma (with exclusion of carcinosarcoma), leiomyosarcomas accounted for 50% of all cases of uterine sarcoma, with ESS for 18.4%, UES for 13.2%, adenosarcomas for 13.2% and other subtypes (mesenchymal chondrosarcoma and rhabdomyosarcoma) for 5.2%. According to FIGO staging, the most frequent stage was stage I (33 patients; 58.9%), followed by stage IV (15 patients; 6.8%). Fifty-three patients (94.7%) underwent surgical resection with 36 patients (64.3%)

receiving complete cytoreduction. In terms of adjuvant therapy, forty-one patients (73.2%) received adjuvant therapy. Fifteen patients (26.8%) had progression of the disease, while 12 patients (21.4%) experienced recurrence.

The 5-year OS was 40% with a median of 40 months while overall 5-year DFS was 34% with a median of 15 months. For carcinosarcomas, the 5-year OS was 54% with a median of 60 months, 5-year DFS was 49% with a median of 35 months. For uterine sarcomas, the 5-year OS was 33% with a median of 25 months, 5-year DFS was 28% with a median of 10 months.

No significant association between OS and DFS with histological subtypes was (p= 0.158 and p = 0.428). For the FIGO staging, classified as early stage (stage I, II) and late stage (stage III, IV), it was found that there was no statistical significance between FIGO staging with OS, but there was significant interest with DFS (OS; 64.1± 9.5 months vs 30.2 ± 7.5 months; p=0.143, DFS; 59.4± 9.8 months vs 22.3 ± 6.9 months; p= 0.020).

Table 1. General characteristics of patients with uterine sarcomas and carcinosarcomas

Characteristics	Numbers (56)	%
Mean age (years) 57.8±10.9		
BMI 25.3±5.0		
Initial symptoms		
AUB	43	76.8%
Mass	7	12.5%
Abdominal pain	2	3.5%
Other	4	7.0%
Surgical type		
Surgical staging	36	64.3%
TAH with BSO	17	30.3%
Tumor removal	1	1.8%
Biopsy	2	3.6%
Pathological diagnosis		
LMS	19	33.9%
Carcinosarcoma	18	32.1%
UUS	5	8.9%
LGESS	4	7.2%
HGESS	3	5.4%
Size of uterine sarcoma (n=38)		
Tumor size ≤ 5 cm.	10	26.3%
Tumor size > 5 cm.	28	73.7%
Surgical staging		
I	33	58.9%
II	2	3.6%
III	6	10.7%
IV	15	26.8%
Adjuvant therapy		

No	15	26.8%
CMT	25	44.7%
RT	9	16.0%
CMT and RT	7	12.5%
Recurrence	15	21.4%
Progression	12	26.8%

BMI: Body Mass index; AUB: Abnormal uterine bleeding; TAH with BSO: Total abdominal hysterectomy with bilateral salpingo-oophorectomy; LMS: Leiomyosarcoma; UUS: Undifferentiated uterine sarcoma; LGESS: Low-grade endometrial stromal sarcoma; HGESS: High-grade endometrial stromal sarcoma; CMT: Chemotherapy; RT: Radiotherapy

Table 2. Clinical characteristics of uterine sarcoma and carcinosarcoma

	LGESS	HGESS	UUS	LMS	other	carcinosarcoma
Number	4	3	5	19	7	18
Age	58.2±5.2	44.7±21.8	64.0±12.7	57.3±8.7	53.6±15.6	60.4±8.1
Menopause	2 (50%)	2 (66.7%)	5 (100%)	12 (63.2%)	4 (57.1%)	17 (94.4%)
BMI	25.9±7.7	21.6 ± 2.1	22.1±3.5	25.9±5.5	25.6±5.1	26.0±4.5
Initial symptoms						
AUB	4 (100%)	3 (100%)	5 (100%)	11 (57.9%)	4 (57.1%)	16 (88.9%)
Mass	0	0	0	4 (21.1%)	1 (14.3%)	2 (11.1%)
Abdominal pain	0	0	0	1 (5.2%)	1 (14.3%)	0
Other	0	0	0	3 (15.8%)	1 (14.3%)	0
Surgical type						
Surgical staging	2 (50%)	2 (66.7%)	2 (40%)	8 (42.1%)	6 (85.7%)	16 (88.9%)
Hysterectomy	2 (50%)	0	3 (60%)	9 (47.2%)	1 (14.3%)	2 (11.1%)
Tumor removal	0	0	0	1 (5.3%)	0	0
Biopsy	0	1 (33.3%)	0	1 (5.3%)	0	0
Tumor size						
≤ 5 cm	2 (50%)	2 (66.7%)	0	3 (15.8%)	3 (42.9%)	NA
> 5 cm	2 (50%)	1 (33.3%)	5 (100%)	16 (84.2%)	4 (57.1%)	NA
FIGO						
I	4 (100%)	1 (33.3%)	3 (60%)	9 (47.4%)	4 (57.1%)	12 (66.7%)
II	0	1 (33.3%)	0	1 (5.3%)	0	0
III	0	1 (33.3%)	2 (40%)	0	0	3 (16.7%)
IV	0	0	0	9 (47.4%)	3 (42.9%)	3 (16.7%)
Adjuvant therapy	1 (25%)	2 (66.7%)	4 (80%)	15 (78.9%)	6 (85.7%)	15 (83.3%)
CMT	1 (25%)	1 (33.3%)	3 (60%)	11 (57.9%)	3 (42.9%)	13 (72.2%)
RT	0	0	3 (60%)	3 (15.8%)	3 (42.9%)	6 (33.3%)
XRT	NA	NA	0	1 (5.3%)	2 (28.6%)	4 (22.2%)
BRT	NA	NA	2 (40%)	1 (5.3%)	0	0
Combined		1 (33.3%)	1 (20%)	1 (5.3%)	1 (14.3%)	2 (11.1%)
Recurrence	0	0	3 (60%)	1 (5.3%)	2 (28.6%)	6 (33.3%)

Progression	1 (25%)	2 (66.7%)	1 (20%)	7 (36.8%)	2 (28.6%)	2 (11.1%)

AUB: Abnormal uterine bleeding; CMT: Chemotherapy; XRT: external radiotherapy; BRT: Brachytherapy; NA: Not applicable

Regarding therapy, adjuvant therapy was provided for both OS and DFS (OS; 67.1 ± 8.9 months vs 23.4 ± 9.2 months; p = 0.010, DFS; 58.2 ± 9.4 months vs 19.8 ± 8.2 months; p= 0.015). In terms of FIGO staging and adjuvant therapy, patients with an early stage of the disease who received adjuvant therapy had a better OS but there was no significant impact on DFS (OS; 73.8 ±10.6 months vs. 31± 12.1 months, p = 0.027, DFS; 67.7 ± 11.2 months vs. 31.7 ± 12.1 months; p= 0.158). In contrast to late stage, adjuvant therapy prolonged both OS and DFS (OS; 37.6± 8.8 months vs. 3.9±1.4 months, p< 0.001, DFS; 30.4 ± 8.7 months vs 2.0 ± 1.3 months, p=0.013), as shown in Figures 1 and 2. There was no significant impact on both OS and DFS when focused on adjuvant radiation and adjuvant chemotherapy individually.

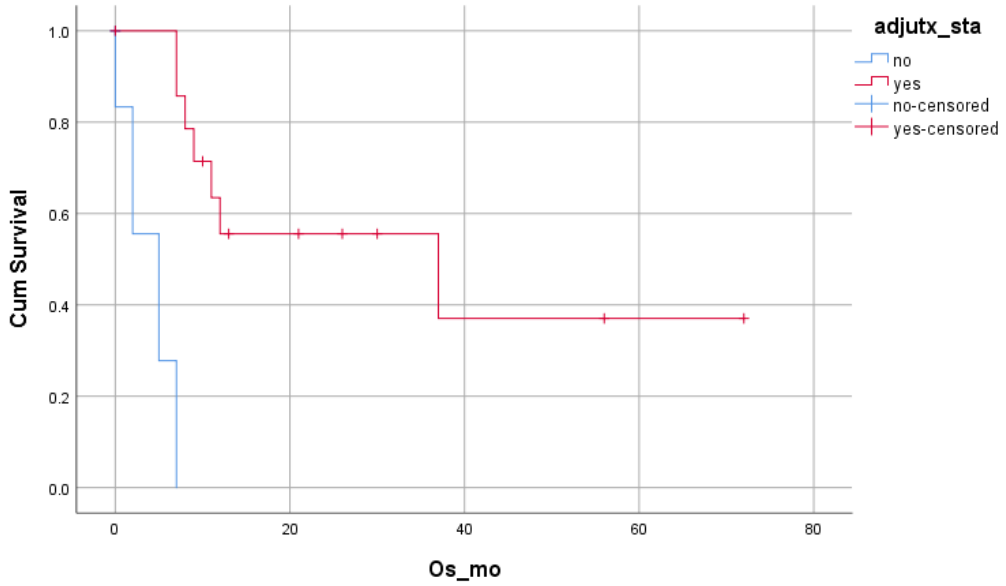

Figure 1. Overall survival (OS) for adjuvant therapy in the late stage (p< 0.001)

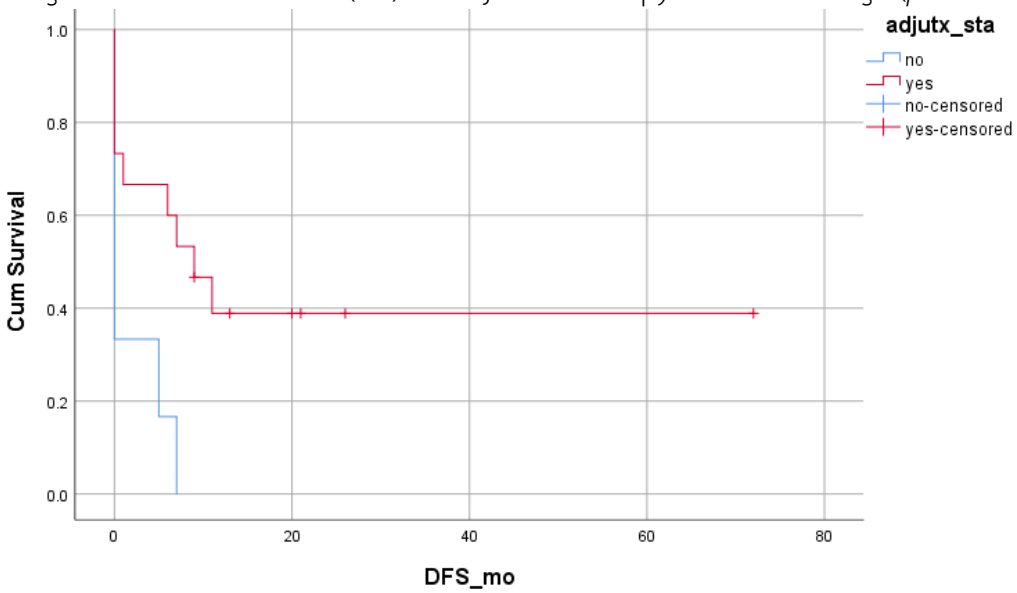

Figure 2. Disease-free (DF) survival for adjuvant therapy in late stage (p = 0.013)

In univariate analysis, OS and DFS were influenced by adjuvant therapy, and DFS alone was influenced by late stage. There was no associated variable on multivariate analysis (Tables 3 and 4).

Table 3. Univariate and multivariate analysis for Overall Survival (OS)

	Univariate		Multivariate	
	HR (95% CI)	p	HR (95% CI)	p
Age				
≤ 40 years	1		1	
> 40 years	0.56 (0.17-1.87)	0.343	0.65 (0.15-2.87)	0.574
BMI				
≤ 25	1		1	
> 25	0.41 (0.21-1.03)	0.059	0.50 (0.19-1.29)	0.154
Histology				
ESS	1		1	
LMS	0.48 (0.16-1.44)	0.189	1.37 (0.34-5.53)	0.655
UUS	0.76 (0.20-2.86)	0.689	2.23 (0.39-12.57)	0.364
Other	0.28 (0.06-1.18)	0.082	0.89 (0.15-5.36)	0.896
Carcinosarcoma	0.28 (0.09-0.94)	0.039	0.78 (0.19-3.24)	0.737
Cytoreduction				
Incomplete	1		1	
Complete	0.47 (0.22-1.01)	0.052	0.95 (0.37-2.47)	0.913
Stage				
Early stage	1		1	
Late stage	1.77 (0.81-3.87)	0.151	1.99 (0.69-5.68)	0.2
Adjuvant therapy				
No	1		1	
Yes	0.27 (0.12-0.60)	0.001	0.24 (0.03-1.99)	0.187
RT				
No	1		1	
Yes	0.55 (0.23-1.31)	0.177	0.68 (0.15-3.15)	0.630
CMT				
No	1		1	
Yes	0.53 (0.25-1.15)	0.107	0.92 (0.16-5.24)	0.920

95% CI; 95% confidence interval; HR: Hazard Risk; RT radiotherapy, CMT: Chemotherapy

Table 4. Univariate and multivariate analysis for disease-free survival (DFS)

	Univariate		Multivariate	
	HR (95% CI)	p	HR (95% CI)	p
Age				
≤ 40 years	1			
> 40 years	0.57 (0.17-1.88)	0.354	0.76 (0.20-2.91)	0.692
BMI				
≤ 25	1			
> 25	0.58 (0.28-1.18)	0.132	0.57 (0.25-1.32)	0.190
Histology				
ESS	1			
LMS	0.83 (0.29-2.35)	0.719	1.06 (0.31-3.58)	0.93
UUS	0.99 (0.26-3.70)	0.989	1.69 (0.34-8.28)	0.519
Other	0.45 (0.11-1.90)	0.278	0.34 (0.15-3.59)	0.702
Carcinosarcoma	0.46 (0.15-1.43)	0.179	0.66 (0.18-2.38)	0.526
Cytoreduction				
Incomplete	1			
Complete	0.49 (0.25-1.00)	0.520	0.91 (0.39-2.10)	0.833
Stage				
Early stage	1			
Late stage	2.20 (1.07-4.52)	0.032	2.18 (0.91-5.20)	0.790
Adjuvant therapy				
No	1			
Yes	0.44 (0.21-0.91)	0.026	0.66 (0.097-4.44)	0.670
RT				
No	1			
Yes	0.50 (0.22-1.17)	0.110	0.49 (0.11-2.21)	0.354
CMT				
No	1			
Yes	0.72 (0.36-1.44)	0.350	0.78 (0.14-4.21)	0.770

95% CI; 95% confidence interval; HR: Hazard Risk; RT radiotherapy, CMT: Chemotherapy

Discussion

Due to the rarity and heterogeneity of uterine sarcomas, there were few data describing these diseases. From our study, the first three most common subtypes were leiomyosarcomas, carcinosarcomas, and endometrial stromal sarcoma. When histologic subtypes within uterine sarcomas were compared, leiomyosarcomas accounted for half of all cases of uterine sarcoma, with ESS accounting for 15-20% of cases. Abnormal uterine bleedings were the most common initial symptoms in all histological types and in all tumor stages. These are similar to the reported data [1-7].

The previous data suggest that histological types may have effects on survival [17]. Due to clinical aspects currently known today, the behavior of low-grade endometrial stromal sarcomas is generally good. They tend to be occurred in younger patients compared to other histologic types. A majority of the patients (65-80%) present at stage I of at the time of diagnosis, with 90% of five-year OS [3,5]. In our study, the mean age of low-grade ESS was 58.5±5.2 years, which is not statistically significant compared to leiomyosarcomas (57.3±8.7 years, p = 0.238) and carcinosarcomas (60.4±8.1 years, p = 0.541). With only three cases, all of them presented at stage I and still alive at the time of followed up. The five-year survival was not reached, and 3-year survival was 71% with a median of 51.6 months. These seemed to have poorer survival than the previously published studies because of limitation of sample size and the time of followed up.

Currently, since tumors have heterogeneity, tumor staging is now the strongest prognostic factor affecting survival. From this present study, it was found that staging had no statistical impact on OS, but there was statistical significance in prolonged DFS. Thus, the conclusion seemed to be that patients with an early stage had better DFS than late stage, but there was no impact on OS.

For other factors, there was no significance in BMI between uterine sarcomas and carcinosarcomas. These results are discordant with the recent studies [4,10-12]. No clinical data on pelvic radiation or prolonged estrogen exposure were available. Only one case with carcinosarcoma in this study had a history of tamoxifen being used due to previous breast cancer. So, it was impossible to come to a conclusion about risk factors for uterine sarcomas and carcinosarcomas.

The five-year OS was 40% with a median of 40 months while overall five-year DFS was 34% with a median of 15 months. These results were quite similar to previous published literature [1-5,16]. But the five years OS of carcinosarcomas (5-year OS; 54% with median of 60 months) seem to be better compared to the previous literatures [15]. Most cases (66.7%) of carcinosarcomas are presented at early stage, with 53% of 5-year OS, which was accordance in literatures. The small sample size and early-stage presentation in most cases result in better prognosis in current study.

Surgery is currently the gold standard and only curative treatment modality in all histological types [2,13-14], while adjuvant therapies are differently performed depends on histological types [8, 14]. Radiotherapy is proven benefit in local regional control while chemotherapy is not recommended [14, 16, 18]. In the current study, almost all patients underwent surgical intervention, and the majority of them received adjuvant therapy. Adjuvant therapy demonstrated a significant impact on both OS and DFS. Due to FIGO staging, both OS and DFS have been prolonged in patients with late stages of the disease when patients received adjuvant therapy, with an unclear effect evident in the early stage. Since this study had a patient who received chemotherapy and radiotherapy alone, and a patient who

underwent combined modality, it is believed that adjuvant therapy may play some important roles in prolonged OS and DFS in patients with advanced stages of the diseases, but the impact on early stage is still inconclusive, while the role of radiation and chemotherapy has been not yet clarified in both survival and local regional control.

This was the first study that described uterine sarcoma in Srinagarind Hospital. All cases in 10 years were included and reviewed, with cooperation between gynecologic oncologist teams and one expert gynecologic pathologist, to provide accurate clinical and pathological data. There were still some limitations. Since it was a retrospective observational study with, a small number of patients, and a short period of follow up time, the data collected are still incomplete. Other important prognostic factors, treatment modality and role of molecular studies should be further studied.

Conclusion

According to the data from uterine sarcomas and carcinosarcoma in Srinagarind Hospital, uterine sarcomas and carcinosarcoma are aggressive tumors. The most common subtype of uterine sarcomas was leiomyosarcomas. Carcinosarcomas are better survival than uterine sarcomas. FIGO staging also have an impact on survival, with patient in early stage had better DFS than late stage, but there was no impact on OS. Adjuvant therapy had impacts on both OS and DFS, particularly in late stages of the disease. However, the prognosis is generally poor outcome.

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ORIGINAL ARTICLE

PREVALENCE OF FACTORS ASSOCIATED WITH CORONARY ATHEROSCLEROSIS IN POPULATION NOT MORE THAN 45 YEARS OLD

Aphichaya Visessaksanti, MD.*, Hataichanok Puengcharoenpong, MD. and Smith Srisont, MD.

Forensic Unit, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University

* Correspondence to: Aphichaya Visessaksanti, MD. Forensic Unit, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University. 270 Rama VI Road, Thung Phayathai, Ratchathewi, Bangkok 10400, Thailand. Contact information: aphichaya1994@gmail.com, charmed_wish@hotmail.com Tel. +662-201-2710

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Abstract

Background: Coronary atherosclerosis is the leading cause of natural death, becoming more common in young people. Several factors are males, underlying diseases (diabetes mellitus, hypertension, dyslipidemia), smoking, alcohol drinking, stimulant abuse, and family history of premature coronary atherosclerosis. A few studies had been conducted on the deceased.

Objectives: The authors aim to identify risk factors and examine postmortem blood cholesterol, which would help prevent the disease.

Materials and Methods: A prospective case-control study was used. The subjects were cases not exceeding 45 years old who had undergone forensic autopsies. Postmortem blood cholesterols were measured. The subjects were classified into those with and without severe coronary atherosclerosis. Questions regarding the risk factors and symptoms were obtained from relatives.

Results: Significant risk factors of coronary atherosclerosis were males (92.3%) and underlying diseases (57.1%). The percentage of other risks were as follows; smoking (61.9%), alcohol consumption (19.0%), stimulant use (10.0%), and family history (5.6%). Symptoms (chest pain, dyspnea, palpitation, loss of consciousness) were noted in 61.5% of the group. Cholesterol levels of both groups were indistinguishable.

Conclusions: Underlying diseases, males, and symptoms were related to premature coronary atherosclerosis.

Keywords: coronary atherosclerosis, postmortem lipid profiles, risk factors, young

Introduction

A medicolegal autopsy, with the purpose of determining the cause of unattended death, is one of the most crucial duties of forensic pathologists. The most common cause of death worldwide is sudden or unexpected natural death [(1)], which means natural death within 24 hours from the onset of symptoms. The most common cause among these is coronary atherosclerosis.

In forensic aspects, an occlusion greater than or equal to 75% of a cross-section of one of the main coronary artery branches can lead to sudden death [(2)]. It is common in age groups more than 60 years old [(3)], but the chance of the younger population suffering the disease has also increased drastically. The definition of coronary artery disease in the young is coronary atherosclerosis in the population under 45 years old [(4)].

Some important risk factors are diabetes mellitus (DM) [(4-8)], hypertension (HT) [(4-6,8)], dyslipidemia (DLP) [(4-6,8)], smoking [(4-8)], history of premature coronary atherosclerosis in the direct relatives [(5,7-8)] (grandparents, parents, and siblings), stimulants (methamphetamine, amphetamine, etc.), alcohol abuse [(8-9)], some underlying diseases such as autoimmune diseases [(4)]

A review of the literature indicated that in patients with premature coronary atherosclerosis, 38.1% had HT, 14.7% had DM [(7)], and 41% had coronary artery disease running in direct relatives [(7)]. Smokers had a five-time higher risk compared to non-smokers [(5,7)]. Males occupied 79-90% of premature coronary atherosclerotic patients, while the percentage dropped to 68.4% when their ages exceeded 45 years. Lastly, heavy alcohol consumption, which was defined as an alcohol intake greater than or equal to 60 and 40 grams per day in males and females, respectively, increased the chance as well [(9)].

However, most previous studies the authors reviewed in the aforementioned paragraphs were conducted on patients, while not many were reviewed on the deceased. Our study aims to investigate risk factors of coronary atherosclerosis in the dead and to analyze postmortem blood total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C). If the cholesterol levels are high and diagnosed with DLP, it can be assumed that premortem cholesterols are unquestionably high. This data would be beneficial for the primary prevention of the deceased's family.

In living persons, coronary angiography will be done only if a patient has signs and symptoms consistent with coronary artery disease. In other words, asymptomatic patients would almost never receive coronary catheterization, and coronary atherosclerosis would not

be discovered. Another advantage of an autopsy was that histological diagnosis of coronary arteries and cardiac muscles could be obtained, while this was impossible from coronary angiography.

Materials and Methods

The authors used a prospective case-control study as a statistical method in this study. Data were obtained from the deceased undergoing medicolegal autopsies performed at the forensic unit, department of pathology, Ramathibodi Hospital and the forensic unit, Ramathibodi Chakri Naruebodindra Hospital starting from December 1, 2020, until January 31, 2022. Autopsy processes began with gross external examination and blood sample collection from the femoral veins in the groin area. Then dissection was done for inspection of internal organs. For the heart, it was weighed, and then the three main coronary artery branches, which included the left anterior descending branch, left circumflex branch, and the right coronary artery, were dissected cross-sectionally, 0.3-0.5 cm in thickness of each cut to determine the degree of luminal occlusion. If present, the occlusion was recorded by percentage per cross-sectional area. The tissues from the brain, heart, lungs, liver, spleen, pancreas, adrenal glands, and kidneys were randomly sampled. In addition, the coronary artery tissues were collected in the cases in which coronary atherosclerosis was related to the cause of death. After that, all tissues were fixed in 10% formalin and stained H&E for microscopic examination.

Data collection

The inclusion criterion is all cases whose age at death did not exceed 45 and had undergone medicolegal autopsies, regardless of the cause of death. Common causes of death in these cases included traffic injuries, natural diseases both related and unrelated to coronary atherosclerosis (for instance, pneumonia, cirrhosis, etc.), suicides, and homicides. In various cases, coronary atherosclerosis was an incidental finding not associated with the cause of death. The exclusion criterion was decomposed corpses since blood samples could not be obtained. Then the cases were classified into two groups; the first group was those with severe coronary atherosclerosis of one or more main coronary arteries: the left anterior descending branch, the left circumflex branch, and the right coronary artery. The coronary artery lumens were divided into quadrants by imaginary lines. Then the total area of occlusion was estimated compared to those quadrants. At least two parties, in a simple manner, one forensic resident doctor and one forensic staff responsible for the case were the ones who indicated the degree of occlusion. If both had divergent opinions, it would be discussed for an agreeable conclusion. Severe coronary atherosclerosis is defined as greater than or equal to 75% occlusion of the arterial cross-section since it is able to lead to sudden death. This group will be referred to as ‘the case group.’ The other group was those with less than 75% occlusion of all coronary arteries, called ‘the control group.’

Additional data were collected using a questionnaire. In most cases, close family members of the deceased, in other words, a spouse, children, parents, or siblings, were the ones who provided the answers. The first question was about the history of DM, HT, and DLP and treatment if available. The second question was about the symptoms related to coronary atherosclerosis, which the deceased might experience at some point in their lives. This included chest pain, dyspnea, palpitation, or sudden loss of consciousness. The third one was about the history of smoking. Regular smoking was defined as smoking greater than or equal to 10 cigarettes per day, and the other group meant non-smokers. The next one was drinking habits, in which the type and amount were inquired about, then calculated. These were classified as regular drinking (greater than or equal to 40 g of alcohol per day almost every day), occasional drinking (not more than once a week and less than 40 grams each), and those who did not drink. The fifth question was about stimulant abuse, e.g., methamphetamine, amphetamine, etc., and the last one was about the history of coronary artery disease in the young, which ran in the family, including grandparents, parents, and siblings

Blood sample collection

From a literature review, there are changes in postmortem lipid profiles [(10)]. While triglyceride and very-low-density lipoprotein cholesterol (VLDL-C) increase, TC, LDL-C, and high-density lipoprotein cholesterol (HDL-C) decrease slightly. Hence, the authors analyze postmortem TC and LDL-C from the bodies. If the postmortem levels exceed dyslipidemia diagnostic criteria, it can be assumed that the premortem levels were actually higher while the deceased was alive.

For blood sample collection, 3-5 ml of blood was drawn from femoral veins and preserved in a clotted blood tube. The samples were stored at room temperature (25 degrees Celsius) not more than 90 minutes before being delivered to the laboratory. The lipid profiles were analyzed in the clinical chemistry laboratory in the department of pathology, faculty of medicine Ramathibodi Hospital. The methods were an enzymatic assay and a selective detergent with enzymatic assay by Abbott® Alinity C, which has been certified ISO 15189 and ISO 15190. The cut points of TC and LDL-C by the laboratory were 200 and 130 mg/dL, respectively.

According to ACC/AHA clinical practical guidelines [(11)], primary hypercholesterolemia is defined as LDL-C 160-189 mg/dL, and LDL-C greater than or equal to 190 mg/dL requires drug intervention regardless of other comorbidities or factors. Thus, the authors used 160 mg/dL as the cut point of hypercholesterolemia diagnosis.

Data analysis

All data were statistically analyzed by SPSS. Chi-square tests were the prime method used in the study. Correlations between severe coronary atherosclerosis and symptoms,

demographic data namely sex, underlying diseases of interest, drinking and smoking habits, drug use, and premature familial coronary artery disease, as well as links between LDL levels and degree of coronary occlusion were all calculated using Chi-square tests. Furthermore, descriptive statistics were used to demonstrate TC and LDL levels.

Ethical statement

Research ethics was approved by the Human Research Ethics Committee, Faculty of Medicine Ramathibodi Hospital, Mahidol University. (COA. MURA2020/1856)

Results

One hundred and fifty-nine cases were included, all of which were Asians. The mean age was 32.3 years ranging from 13-45 years. There were 135 males and 24 females. The case group consisted of 52 cases (32.7%) which comprised 48 males (92.3%) and four females (7.7%) with a mean age of 37.5 years. The remaining, in other words, the control group, accounted for 107 cases, which were 87 males (81.3%) and 20 females (18.7%) with a mean age of 30.3 years. A total number of blood samples were 48, of which 10 cases (32.7%) possessed severe coronary atherosclerosis and the rest, 38 cases (67.3%), did not. Statistical analysis exhibited a greater chance of males having coronary atherosclerosis than females statistically non-significantly (P-value = 0.069). The percentages of each gender were 92.3% and 7.7%, respectively. All considered factors were listed in Table 1.

Table 1. Risk factors associated with premature coronary atherosclerosis

Parameter	case (%)	control (%)	P-value
Sex (male)	92.3	81.3	0.69
Underlying diseases	57.1	6.1	0.002
Smoking	61.9	50.5	0.347
Alcohol consumption	19.0	11.2	0.801
Stimulant use	10.0	11.2	1.000
Family history	5.6	4.8	1.000

Regarding underlying diseases, there were 86 out of 159 samples (54%) with unknown history of DM, HT, and DLP. Forty-five unknown cases belonged to the case group; in other words, 86.54% of the group. Another 41 cases were that of unknown health history with non-severe coronary atherosclerosis, accounting for 38.31%. It was due to the absence of health

check-ups, the deceased's health was unknown to the families, or the illness was unrecognized to the families.

The unknown data was not included in the statistical calculation. Thus, only 73 cases were calculated; 7 out of 73 cases were of the case group. Four cases from these 7 (57.1%) had one of the diseases of interest, while only 6.1% of the control group had one or more underlying diseases. These data were statistically significant (P-value < 0.005). In addition, some of those with known underlying conditions were on treatment while some were not or had poor compliance. The subsequent risk factor studied was a smoking habit. Cases with unknown smoking history accounted for 47 out of 159 cases (29.56%). Thirty-one of them were of the case group (59.62%), and 16 did not (14.95%). One hundred and twelve cases with known history consisted of 21 and 91 cases, respectively, with and without severe coronary occlusion. It was revealed that the former group had an insignificant larger percentage of smokers (P-value 0.347).

For a history of alcohol consumption, there were 45 out of 159 cases with unknown drinking habits or 28.30%. Thirty-one cases belonged to the case group (59.62%), and the remaining 14 were of the control group (13.08%).

One hundred and fourteen cases with known history were classified into three groups according to the amount of alcohol consumed; regular drinking (greater than or equal to 40 g of alcohol per day, almost every day), occasional drinking (not more than once a week and less than 40 grams each), and those who did not drink. Occasional drinkers made up the most considerable fraction. Drinking habits were insignificantly unrelated to risks of developing the disease (P-value 0.801).

There were 50 cases with unknown history of stimulant abuse, accounting for 31.45%. Thirty-two subjects had severe coronary atherosclerosis (61.54%), and 18 cases belonged to the control group (16.82%). Therefore, 109 known-history cases were calculated. It was shown that stimulant abuse and risks of developing premature coronary atherosclerosis were unrelated. The percentages of drug abuse among case and control groups were not different, 10.00% and 11.20%, respectively (P-value 1.000).

The last risk factor studied, a history of premature coronary atherosclerosis in the family, had an unknown history for 57 cases, accounting for 35.85% of 159 cases. The case group owned 34 unknown cases, or 65.38%, while the remaining 23 cases belonged to the control group (21.50%).

One hundred and two cases with known family history were computed and revealed no correlation between the disease and the family history. Only 5.6% of the deceased with severe coronary atherosclerosis had a history of the disease running in the family. (P-value 1.000)

The total blood sample obtained was 48 samples, ten from the case group (20.8%) and 38 from the control group (79.2%). The levels of TC and LDL-C of the two groups showed

no difference. The means of TC of the case group and the control group were 168.40 and 173.87 mg/dL, respectively, and those of LDL-C were 108.70 and 113.50 mg/dL, respectively.

Since all guidelines the authors reviewed recommended drug intervention using one or more lipid-lowering agents when LDL-C level is equal to or exceeds 190 mg/dL, the authors also classified the data using the number. There were 2 cases in which LDL-C greater than or equal to 190 mg/d (192 and 233 mg/dL), which were one male and one female each. None of them had severe coronary atherosclerosis. Among 36 cases with LDL less than 190 mg/dL, 10 cases (21.7%) had severe coronary occlusion, and the remaining 36 cases (78.3%) did not have severe coronary atherosclerosis. The latter comprised 34 males (73.9%) and 12 females (26.1%). None of these aforementioned numbers were statistically significant.

Moreover, the authors used LDL-C 160 mg/dL as the cut point according to ACC/AHA guidelines. The results were as follows:

In the group with LDL-C greater than or equal to 160 mg/dL, there were five males (71.4%) and two females (28.6%) out of 7 cases. Only one of those was a male who had severe coronary atherosclerosis with LDL-C 187 mg/dL. The remaining 6 cases were five males and one female. The highest and lowest LDL-C are 233 and 179 mg/dL, respectively, with the mean LDL-C of 190 mg/dL. For the group with LDL-C less than 160 mg/dL, there were 30 males (73.2%) and 11 females (26.8%). The highest and lowest levels were 154 and 7 mg/dL, respectively. None of these were statistically non-significant. (Tables 2 and 3)

Table 2. LDL-C using 160 as the cut point

LDL-C (mg/dL)	<160	≥160	Total
Cases	9 (22.0%)	1 (14.3%)	10 (20.8%)
Control	32 (78.0%)	6 (85.7%)	38 (79.2%)
Total	41	7	48

P-value 1.000 LDL-C: low-density-lipoprotein cholesterol

Table 3. LDL-C using 160 and 190 mg/dL as cut points

LDL-C (mg/dL)	<160	160-189	≥190	Total
Cases	9 (22.0%)	1 (20.0%)	0 (0.0%)	10 (20.8%)
Control	32 (78.0%)	4 (80.0%)	2 (100.0%)	38 (79.2%)
Total	41 (100.0%)	5 (100.0%)	2 (100.0%)	48 (100.0%)

P-value 1.000 LDL-C: low-density-lipoprotein cholesterol

The mean age of the groups which met and did not meet LDL-C diagnostic criteria was 34.7 years with SD 5.37 and 32.8 years with SD 8.54, respectively. The P-value of the mean age difference was 0.572, which was non-significant.

The signs and symptoms of coronary atherosclerosis were, similar to other data, unknown to the families of 83 from 159 cases, or 52.20%. 39 cases belonged to the case group (75%), and 44 cases were that of the control group (44.12%).

The symptoms of coronary atherosclerosis the authors included in the study were at least one of the followings; chest pain, dyspnea or shortness of breath, sudden loss of consciousness, and palpitation. A more significant number of the case group had symptoms than the control group significantly. 61.5% of the case group had manifested at least one symptom, while only 19.0% of the control group had reported the symptoms (P-value = 0.004). (Table 4)

Table 4. Symptoms related to coronary atherosclerosis

Symptoms	No	Yes	Total	Unknown
Cases	5 (38.5%)	8 (61.5%)	13 (100.0%)	39
Control	51 (81.0%)	12 (19.0%)	64 (100.0%)	44
Total	56	20	76	83

P-value 0.004

Discussion

This research aims to study the risk factors of coronary atherosclerosis in a population not more than 45 years old. The data were obtained and compared between the deceased with greater than or equal to 75% occlusion of one or more coronary arteries and those with less than 75% occlusion of all three coronary branches. The information gathered was the history of underlying diseases of interest (DM, HT, DLP), drinking habits, smoking habits, drug abuse, the history of premature coronary artery disease in the family, and the symptoms related to coronary atherosclerosis.

The insignificant risk factors from this study were as follows; Smoking and a family history of premature coronary artery disease did not insignificantly raise the risks of developing coronary atherosclerosis in the young, which was contrary to the studies by Shah, N. et al. (2019) [(5)] and Malakar, A. K. et al. (2019) [(6)]. Alcohol consumption did not increase the risk as well, inconsistent with the study by Rehm, J. and Roerecke, M. (2017) [(9)]. At the same time, the three mentioned underlying diseases increased the risk of premature coronary atherosclerosis, concordant with the research by Poorzand, H. et al. (2019) [(4)].

This study and previous studies showed that DM, HT, and DLP were strongly related to coronary atherosclerosis. Nevertheless, numerous cases had never had a health check-up, possibly diseased but never realized. Thus, promoting healthy lifestyles and an early disease screening were essential. Consequently, there would be fewer complications from the illness. Furthermore, less expense would be invested in curing chronic diseases. Those all would create the overall well-being of the population.

The study showed that more cases from the case group had a history of smoking than the control group, yet without statistical significance. Possible reasons were various intervals of smoke exposure and whether the deceased were second-hand smokers.

There was a likelihood that some history regarding smoking, drinking, and substance abuse was concealed by the families due to moral and legal concerns. Moreover, another possible reason was that the deceased had stopped using stimulants before death. Various substances could not be detected in case of no recent or current abuse.

The limitations of this research were the small sample size and a large number of cases with an unknown history. The data of underlying diseases had the highest percentage of unknown data, followed by signs and symptoms, family history, drug abuse, smoking habits, and drinking habits. Some possible reasons for the latter were numerous people who lived in the areas under Ramathibodi Hospital's responsibility were from the rural areas and lived alone. Those individuals never got a health check-up or did but never informed the families. Recall bias was also responsible since the families might not recall the history of the deceased accurately.

One possible reason for unknown underlying diseases was that those people never got a health check-up and did not realize their health conditions. Hence, they were unaware of health care, which led to premature coronary atherosclerosis. Nevertheless, the autopsies revealed that a number of cases with severe coronary atherosclerosis had signs of end-organ damage related to the diseases of interest. Cardiomegaly, nephrosclerosis, diabetic nephropathy, e.g. hyaline arteriolosclerosis and Kimmelstiel-Wilson nodules, and cerebral arteriolosclerosis are exhibited in some coronary atherosclerotic cases.

The unknown history of premature coronary atherosclerosis inherited in the family was likely to be the same as those of the deceased. Or else a late family member did not have an autopsy performed, and coronary artery occlusion was never detected.

Besides the deceased's information, the authors also examined the level of postmortem TC and LDL-C to compare the case and control groups. There are several clinical practice guidelines relating to dyslipidemia with different diagnostic criteria for dyslipidemia. However, every guideline states that LDL-C greater than or equal to 190 mg/dL requires drug intervention regardless of other risk factors.

According to ACC/AHA clinical practice guidelines [(11)], dyslipidemia requiring intervention or drug treatment varies from person to person. Aside from LDL-C level, several

risk factors such as age, comorbidities, and family history were considered. However, the research could not establish the data since there were a significant number of cases with an unknown history.

For further research, more cases and blood samples should be obtained. Questionnaires with more coverage would be considered, for instance, a more detailed history of alcohol consumption, smoking habits, and history of being a second-hand smoker. In addition, the authors might guarantee informants that all data would be confidential. No data, even those concerning laws and ethics, would not be revealed and traced back to the deceased and families.

Conclusion

This study reveals that premature coronary atherosclerosis risk factors are male, underlying DM, HT, and DLP. Symptoms listed as chest pain, dyspnea, sudden loss of consciousness, and palpitation are clearly related to the disease. In contrast, alcohol consumption, smoking, and stimulant abuse were not associated with the development of coronary atherosclerosis.

Postmortem TC and LDL-C levels demonstrate no correlation to coronary atherosclerosis.

Some noticeable limitations of the study were the large number of cases with unknown history and the small sample size.

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CASE REPORT

CYTODIAGNOSIS OF MICROFILARIA AT UNUSUAL SITES - CASE SERIES

DR. ASIF SHAIKH, DR. (PROF.) NISHI TANDON, DR. REETA CHAUDHARY, DR. AMRISHA JAISWAL, DR. MONIZA ILYAS and DR. (PROF.) NIRUPMA LAL

DEPARTMENT OF PATHOLOGY, ERA'S LUCKNOW MEDICAL COLLEGE U.P INDIA.

* Correspondence to: DR. (PROF.) NISHI TANDON, Postal Address- 3rd floor, Pathology Department, Era's Lucknow Medical College, Hardoi sarfarazganj road, Lucknow, Uttar Pradesh 226003. Phn no. 9452296953 Email Id- drnishitandon@gmail.com

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Abstract

Filariasis is a major health problem in tropical countries including India. The disease is endemic in many states of India. Filariasis is a mosquito borne parasitic infection. It is also referred as elephantiasis. It mostly affects the lymphatic system and may lead to debilitating condition if goes undiagnosed. The burden of filariasis is high in endemic regions and vast majority of population is carrier. Although microfilaria in fine needle cytology is not a common finding, it was observed in various cytological materials i.e., aspirated materials from lymph node, breast lump, cutaneous swelling, bone marrow, bronchial aspirate, nipple discharge, ascitic fluid, pleural fluid, pericardial fluid, ovarian cyst fluid and cervicovaginal smears. Here we present rare cases of filariasis with unusual sites of prestation. It highlights 2 important facts- i) presence of microfilariae in peripheral blood does not always corroborate with presentation/ diagnosis of filariasis in endemic areas. ii) Any patient presenting with swelling/lump in endemic zones, filariasis should always be kept as differential diagnosis.

Keywords: FNAC, Microfilaria, Nematode, Lymphatic system

Introduction

Filariasis is one of the endemic health issues worldwide. It has high prevalence in tropical and subtropical regions. Filariasis is a major health problem in India. The disease is endemic in some regions including Uttar Pradesh, Andhra Pradesh, Odisha, Gujarat, Bihar, Jharkhand, Tamil Nadu, Kerala [(1)]. In India Lymphatic filariasis is caused mainly by two species of nematodes: Wuchereria bancrofti and Brugia Malayi. Female mosquito Culex Quinquefasciatus as vector [(2)]. The adult filarial worms reside in lymphatic system, from where the gravid female releases large number of Microfilaria which may pass through the thoracic duct and pulmonary capillaries into the peripheral blood and occasionally Microfilaraemia may be found in the infected patients. Microfilariae circulating in peripheral blood stream show nocturnal periodicity which makes the parasite difficult to demonstrate on routine investigations. Disease outcome of filarial infection varies from person to person. In endemic areas, majority of population remain asymptomatic; despite of high Microfilarial density in peripheral blood. Blocking of lymphatic channels and nodes by adult worms causes typical presentations of filariasis. Most common clinical presentation of filariasis are acute lymphangitis and lymphadenitis, chronic lymphadenitis, lymphadenopathy, lymphangiovarix,

oedema of limbs, hydrocoele, and tropical pulmonary eosinophilia [(2)]. Clinical presentation may be determined by the sites affected. Most frequently, the lymphatic system of the lower limbs, retroperitoneal tissues, mammary glands, spermatic cord and epididymis is involved [(3)]. A heavy parasite (microfilaria) load may appear in body fluid like including blood, urine, chyle, scrotal aspirates, occasionally thyroid & vaginal aspirates. FNAC place a pivotal role in prompt recognition of disease.

Material and method

This study was undertaken to analyze cytological aspirates of cases where incidentally we found microfilaria from different body sites. We got 15 cases which included 4 breast lump, 4 vaginal pap smear, 3 thyroid swelling, 4 arm cysts swelling.

Lesions from unusual sites e.g., including lymph nodes, salivary glands, thyroid swelling, Pleural fluid, Vaginal Smear, and breast have been included in the study. The cases were correlated with relevant history, physical examination findings and other available investigations. FNAC was performed using 23-gauge needle and 10 ml sterile syringe. Smears of the aspirated material and fluid were stained with Leishman Giemsa (LG) stain. Some of the FNAC smears were air dried for Leishman Giemsa stain and others fixed in 95% ethanol and stained with Papanicolaou (PAP) stain and mounted in DPX & cover with cover slip. The smears were examined under microscope in our Cytology Laboratory of our Department of Pathology.

Case no.	Age/Sex	Types of Specimen	Clinical Presentation	Cytological Findings		Associated Malignancy
				Microfilaria	Inflammatory Infiltrates	
1	40/F	Aspirate, Rt. Breast	6x3 cm, Breast Swelling	+	L+, E+	Nil
2	38/F	Aspirate, Rt. Breast	5x2 cm, Breast Swelling	+	L+, E+	Nil
3	30/F	Aspirate, Rt. Breast	2x2 cm, Breast Lump	+	Nil	Nil
4	48/F	Aspirate, Lt. Breast	4x3 cm, Breast Lump	+	L+, E+	Nil
5	80/F	Cervical Aspirate	Discharge/Bleeding P/V	+	L+, P+, E+	Atypical Cells
6	61/F	Cervical Aspirate	Discharge P/V	+	P+	Nil
7	68/F	Cervical Aspirate	Discharge P/V	+	P+	Nil
8	45/F	Cervical Aspirate	Discharge P/V	+	P+, E+	Nil
9	20/M	Aspirate, Lt. Arm	2x1.5 cm Mid Arm Swelling	+	L+, P+, E+	Nil

10	35/M	Aspirate, Rt. Arm	3.5x2 cm Antero-Lateral Aspect of right Arm	+	L+, P+, E+	Nil
11	45/F	Aspirate, B/L Axilla	Bilateral Axillary Swelling	+	L+ E +granulomas	Nil
12	25/F	Aspirate, Rt. Arm	3x2 cm, Right hand Swelling	+	L+, P+, E+	Nil
13	35/F	Aspirate, Thyroid	3.5x3.5 Midline Neck Swelling	+	L+, P+, E+	Nil
14	44/F	Aspirate, Thyroid	4x2 cm Midline Neck Swelling	+	Nil	Nil
15	58/F	Aspirate, Thyroid	6x4 cm, Right side neck Swelling	+	L+, E+, granulomas	Nil

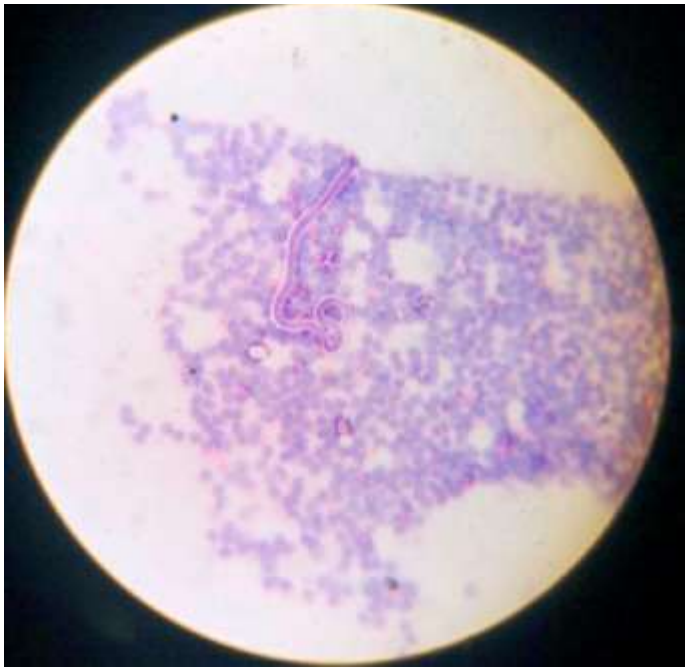

Fig 1. H&E; 100X: Case 1(breast): Microfilaria along with ductal epithelial cells.

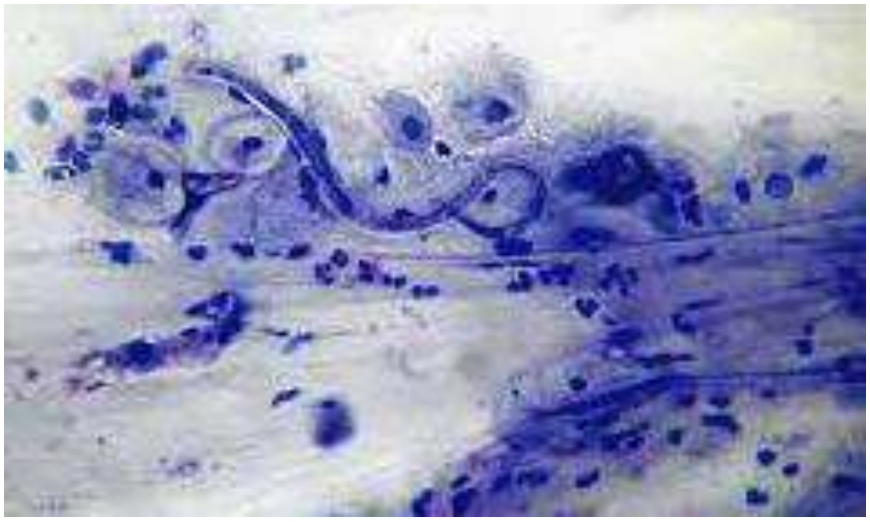

Fig 2. H&E;400X: Case 2(vaginal pap): Microscopic examination reveals presence of predominant parabasal epithelial cells along with intermediate epithelial cells with mild inflammatory infiltrate. Smears showed presence of many Microfilarial larvae having sheath, which was projected slightly beyond the body of larvae. The central axis of larval body contained nuclei, which appeared as granules and were present at the tip of microfilaria of W.bancrofti from the other sheathed larvae.

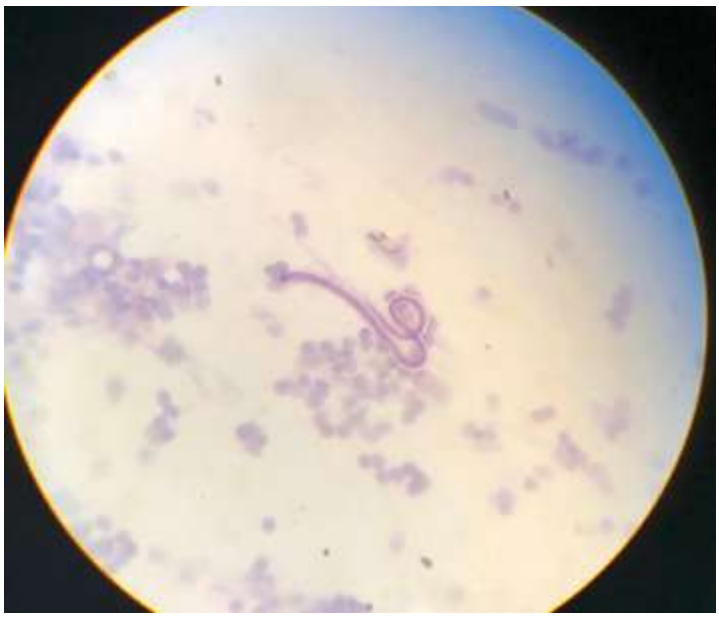

Fig 3. H&E;100X: Case 5(Thyroid swelling): Smear showed inflammatory infiltrate consisting of lymphocytes, eosinophils, polymorphs and few histiocytes.

Smears also shows fair no. of microfilariae sheath as well as non-sheath forms against proteinaceous background.

A wet smear was also made out of it as Chylous fluid raised the suspicion for parasitic lymphatic involvement which revealed wriggling parasitic worm.

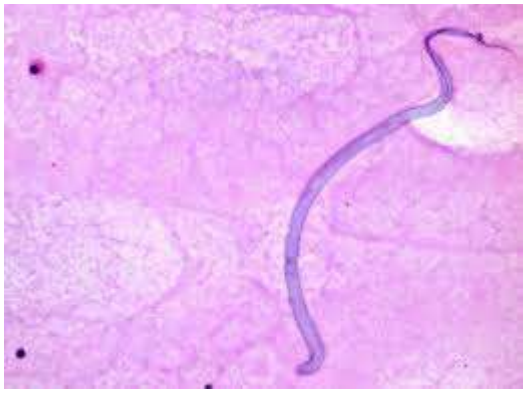

Fig4. H&E;400X: Case 3(Arm cyst swelling): Microfilaria on proteinaceous background

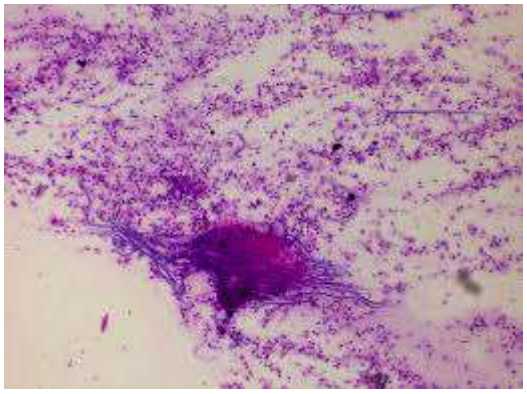

Fig 5. H&E; Scanner: Case 4(Arm cyst swelling): Tangled microfilaria showed only empty sheaths without nuclei against proteinaceous background with dense inflammatory infiltrates.

Discussions

Filariasis is a major public health problem in tropical countries, including India. It is caused by nematodes Wuchereria bancrofti, Brugia Malayi, B. Timori, Loa-loa, Onchocerca volvulus, M.Ozzardi, Mansonella Perstans[4]. Nematodes belongs to the superfamily Filarioidea. Filarial worms infect human and they are definite host while female mosquito are intermediate host. Species acting as vectors may vary in different geographical areas. Wuchereria Bancrofti (90%) and Brugia Malayi (10%) are the most common species causing filariasis in India [5]. This parasite most frequently resides in lymph nodes, lymphatic system. Other filariae mature in the skin and subcutaneous tissues, where they induce nodule formation and dermatitis. The disease can also spread to lungs, pleural, pericardial, ascitic fluid, ovarian cyst fluid, breast lump, bone marrow, bronchial aspirate, thyroid, parotid and gallbladder [5-6]. Few authors have reported microfilariae in breast lumps on FNAC smears [5-7]. Cytological smears demonstrating filarial parasite from many

unusual sites is an incidental finding [8-9] and they have rarely been detected in association with neoplastic lesions in cytological smears.

The acute phase is characterized by fever, lymphadenitis, lymphangitis, epididymo-orchitis, and funiculitis. Headache, nausea, anorexia, backache, muscle pain, insomnia, urticarial rash, malaise and fatigue are common complaints. Eosinophilia and microfilaremia are common in acute phase. Chronic stage of bancroftian filariasis is characterized by lymphadenopathy, lymphedema, hydrocele, and elephantiasis. Adult worms live in the lymphatic channels of the definitive host and microfilaria is released and circulated in the peripheral blood. Female W. Bancrofti measures 80–100 × 0.25 mm and the male 40 × 0.1 mm. The laboratory findings used for diagnosis of filariasis include demonstration of microfilaria in peripheral blood smear, in fluids, biopsy specimens, and by serological tests.

In these situations, the diagnosis remains merely incidental as in case no -1. Though in India is an endemic zone, it is rare to report microfilaria in breast lump. Our case presented with single, firm, nontender, mobile, unilateral breast lump. Most commonly upper outer quadrant is involved yet rarely central or Periareolar nodules may present. No inflammatory changes were present, which made it clinically indistinct from fibroadenoma due to its chronic presentation and failure to resolve instead on antibiotic therapy. Microfilaria was not detected in blood smear. On FNAC, the diagnosis was confirmed by the detection of microfilaria along with inflammatory infiltrate. The detection of microfilaria in vagina (case no-5-8) is also an unusual finding. On reviewing the literature; Walter A et al, Fitz hugh VA, Punia RS et al have reported similar finding [10-12]. Genital morbidity in females is very rare. Patient presented with bleeding per vagina. Clinical evidence of filariasis was absent. Blood smears were negative for microfilariae of W. Bancrofti whereas PAP smear demonstrated Microfilaria with Polymorph, Lymphocytes and epithelioid cells.

Association of bancroftian microfilaria in thyroid nodule is probably the first reported case at our institution. This association is presumed to be purely incidental. Microfilaria rarely present in thyroid (case no-13-15). Patient in euthyroid state presented with solitary thyroid nodule. Aspiration of the swelling demonstrated microfilaria, few histiocytes and lymphocytes.

Almost all the cases had no other relevant symptoms. On examination no lymphadenopathy or organomegaly was palpable. No abnormality was found on local urogenital examination. Routine hemogram was within normal limits. The peripheral blood smear examination did not report any parasite. On microscopy showed a sheathed parasite with characteristic morphology of a microfilariae

The four cases presented as arm cyst (case no-9-12). Filariasis rarely presents as cystic swelling [10,13]. Eosinophilia resulting from filariasis may be attributed mistakenly to coexistent pathologies [14]. The difference in the swelling type (nodular or thickened cord like) and nature of aspirates (clear or chylous) could be owing to extravasation in the first case. Findings of the couple of cases also varied with respect to peripheral blood eosinophilia and FNA smear, that

is Microfilarial morphology and the associated inflammatory response. Peripheral blood smear examination is a useful but relatively less sensitive tool to diagnose filariasis. Eosinophilia, although relatively common, and non-specific, as in case 9,10 and 12, is not invariable [15]. Cellular background of granulocytes and giant cells in the second case versus clean background of the first case may be the result of host tissue response because of lymphatics involved. The cytology of the filarial infestation can reveal microfilaria with or without adult worms and may associated eosinophils, neutrophils and mononuclear cells [10,13]. Both cases had different presentation in terms of type of swelling, nature of aspirate i.e., clear aspirate while other was chylous aspirate. Variation in findings were also present with respect to peripheral eosinophilia, Microfilarial morphology and the associated with inflammatory response on FNA smear. To diagnose filariasis, presence of eosinophilia is not mandatory, like in case 5. In all 15 cases in the present study, microfilariae of W. Bancrofti were detected, as suggested by their typical morphologic appearance. The cephalic space at the anterior end is 5-7 microns long and the anterior nuclei are side by side. The caudal space at the pointed posterior end is 5-15 micron long and the terminal nuclei are elongated. Presence of ova and adult worms of filarial organism in cytological smears may or may not be associated with simultaneous presence of microfilaria.

The main aim of the study is to highlight that patient presenting with any swelling in endemic areas, filariasis should always be considered as a differential diagnosis. However, portal of entry of microfilaria in end sites is still a speculation. Most of the authors have explained that as microfilaria circulate in vasculature & lymphatic system. Whenever there is Vasculolymphatic obstruction due to adult worms, inflammation, injury, fibrosis or tumour, these extravasate to extra lymphatic space, they appear in tissue fluid or shed off in surface material. In malignancy increased vasculature also causes increased deposit of microfilaria to these sites. Migration to aberrant sites inside host immune reaction resulting in swelling. This study also emphasizes the magnitude of screening smears for parasites even in the absence of clinical presentation and also peripheral eosinophilia are not compulsion to diagnose microfilaria.

Conclusion

The association of Microfilariasis should be kept in mind in endemic area such as India. FNAC can be a very sensitive and cost-effective tool for the detection of helminthic etiology in unexplained lymphadenopathy or parotitis after exclusion of tuberculous cause. Careful screening of cytologic smears should be done for detection of coexistent Microfilarial infestation with other benign or malignant pathology to detect the hidden burden of Microfilarial diseases in tropical country like India and provide accurate treatment.

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APPENDIX 1
INFORMATION FOR AUTHORS

All authors listed in a paper submitted to Asian Archives of Pathology (AAP) must have contributed substantially to the work. It is the corresponding author who takes responsibility for obtaining permission from all co-authors for the submission. When submitting the paper, the corresponding author is encouraged to indicate the specific contributions of all authors (the author statement, with signatures from all authors and percentage of each contribution can be accepted). Examples of contributions include: designed research, performed research, contributed vital new reagents or analytical tools, analysed data, and wrote the paper. An author may list more than one type of contribution, and more than one author may have contributed to the same aspect of the work.

Authors should take care to exclude overlap and duplication in papers dealing with related materials. See also paragraph on Redundant or Duplicate Publication in "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" at http://www.icmje.org/index.html.

The submitted manuscripts will be reviewed by the members of the Editorial Board or the expert reviewers. At the discretion of the Editorial Board, the manuscripts may be returned immediately without full review, if deemed not competitive or outside the realm of interests of the majority of the readership of the Journal. The decision (reject, invite revision, and accept) letter will be coming from the Editorial Board who has assumed responsibility for the manuscript's review. The editor's decision is based not just on technical merit of the work, but also on other factors such as the priority for publication and the relevance to the Journal's general readership. All papers are judged in relation to other submissions currently under consideration.

Categories of Manuscripts

1. Letters to the Editor

The letters to the editor are the reactions to any papers published in AAP. These letters will be reviewed by the Editorial Board and sent to the authors of the original paper with an invitation to respond. Letters and eventual responses will be published together, when appropriate.

- *Word Count: 300 – 500 words (excluding references and figure or table legends)*
- *Abstract: Not required*
- *References: Maximum of 10*
- *Figure or Table: Maximum of 1 (if needed)*

2. Original Articles

The original articles are the researches describing the novel understanding of anatomical pathology, clinical pathology (laboratory medicine), forensic medicine (legal medicine or medical jurisprudence), molecular medicine or pathobiology. Systematic reviews, meta-analyses and clinical trials are classified as articles. The articles should be clearly and concisely written in the well-organised form (see ***Organisation of Manuscripts***): abstract; introduction; materials and methods; results; discussion; and conclusions. The manuscripts that have passed an initial screening by the Editorial Board will be reviewed by two or more experts in the field.

- *Word Count: 3,000 – 5,000 words (excluding abstract, references, and figure or table legends)*
- *Structured Abstract (see* ***Organisation of Manuscripts****): 150 – 200 words*
- *References: Maximum of 150*
- *Figures or Tables: Maximum of 6*

3. Review Articles

The review articles are generally invited by the Editor-in-Chief. They should focus on a topic of broad scientific interest and on recent advances. These articles are peer-reviewed before the final decision to accept or reject the manuscript for publication. Therefore, revisions may be required.

- *Word Count: 3,000 – 5,000 words (excluding abstract, references, and figure or table legends)*
- *Unstructured Abstract: 150 – 200 words*
- *References: Maximum of 150*
- *Figures or Tables: Maximum of 4*

4. Case Reports

AAP limits publication of case reports to those that are truly novel, unexpected or unusual, provide new information about anatomical pathology, clinical pathology (laboratory medicine) or forensic medicine (legal medicine or medical jurisprudence). In addition, they must have educational value for the aforementioned fields. The journal will not consider case reports describing preventive or therapeutic interventions, as these generally require stronger evidence. Case reports that involve a substantial literature review should be submitted as a review article. The submitted case reports will undergo the usual peer-reviewed process.

- *Word Count: 1,200 – 2,000 words (excluding abstract, references, and figure or table legends)*
- *Unstructured Abstract: 150 – 200 words*

- *References: Maximum of 20*
- *Figures or Tables: Maximum of 4*

5. Case Illustrations

Case illustrations are aimed to provide education to readers through multidisciplinary clinicopathological discussions of interesting cases. The manuscript consists of a clinical presentation or description, laboratory investigations, discussion, final diagnosis, and up to 5 take-home messages (learning points). Regarding continuous learning through self-assessment, each of the case illustrations will contain 3 – 5 multiple choice questions (MCQs) with 4 – 5 suggested answers for each question. These MCQs are placed after the final diagnosis and the correct answers should be revealed after the references. The questions and take-home messages (learning points) are included in the total word count. The manuscripts that have passed an initial screening by the Editorial Board will be reviewed by two experts in the field.

- *Word Count: 1,000 – 2,000 words (excluding references and figure or table legends)*
- *Abstract: Not required*
- *References: Maximum of 10*
- *Figures: Maximum of 2*
- *Tables: Maximum of 5*

6. Technical Notes

The technical notes are brief descriptions of scientific techniques used in the anatomical pathology, clinical pathology (laboratory medicine), forensic medicine (legal medicine or medical jurisprudence), molecular medicine or pathobiology. The submitted manuscripts are usually peer-reviewed.

- *Word Count: Maximum of 1,000 words (excluding references and figure or table legends)*
- *Abstract: Not required*
- *References: Maximum of 5*
- *Figures or Tables: Maximum of 2*

Organisation of Manuscripts

1. General Format

The manuscripts written in English language are preferable. However, Thai papers are also acceptable, but their title pages, abstracts, and keywords must contain both Thai and English. These English and Thai manuscripts are prepared in A4-sized Microsoft Word documents with leaving 2.54-cm (1-inch) margins on all sides. All documents are required to be aligned left and double-spaced throughout the entire manuscript. The text should

be typed in 12-point regular Times New Roman font for English manuscript and 16-point regular TH SarabunPSK font for Thai manuscript.

The running titles of English and Thai manuscripts are placed in the top left-hand corner of each page. They cannot exceed 50 characters, including spaces between words and punctuation. For the header of English paper, the running title will be typed in all capital letters. The page number goes on the top right-hand corner.

Footnotes are not used in the manuscripts, but parenthetical statements within text are applied instead and sparingly. Abbreviations should be defined at first mention and thereafter used consistently throughout the article. The standard abbreviations for units of measure must be used in conjunction with numbers.

All studies that involve human subjects should not mention subjects' identifying information (e.g. initials) unless the information is essential for scientific purposes and the patients (or parents or guardians) give written informed consent for publication.

2. Title Page

The title page is the first page of the manuscripts and must contain the following:

- The title of the paper (not more than 150 characters, including spaces between words)
- The full names, institutional addresses, and email addresses for all authors (If authors regard it as essential to indicate that two or more co-authors are equal in status, they may be identified by an asterisk symbol with the caption "These authors contributed equally to this work" immediately under the address list.)
- The name, surname, full postal address, telephone number, facsimile number, and email address of the corresponding author who will take primary responsibility for communication with AAP.
- Conflict of interest statement (If there are no conflicts of interest for any author, the following statement should be inserted: "The authors declare that they have no conflicts of interest with the contents of this article.")

3. Abstract

A structured form of abstract is used in all Original Article manuscripts and must include the following separate sections:

- *Background: The main context of the study*
- *Objective: The main purpose of the study*
- *Materials and Methods: How the study was performed*
- *Results: The main findings*
- *Conclusions: Brief summary and potential implications*

- *<u>Keywords</u>: 3 – 5 words or phrases (listed in alphabetical order) representing the main content of the article*

4. Introduction

The Introduction section should clearly explain the background to the study, its aims, a summary of the existing literature and why this study was necessary or its contribution to the field.

5. Materials and Methods

The Materials and Methods section must be described in sufficient detail to allow the experiments or data collection to be reproduced by others. Common routine methods that have been published in detail elsewhere should not be described in detail. They need only be described in outline with an appropriate reference to a full description. Authors should provide the names of the manufacturers and their locations for any specifically named medical equipment and instruments, and all chemicals and drugs should be identified by their systematic and pharmaceutical names, and by their trivial and trade names if relevant, respectively. Calculations and the statistical methods employed must be described in this section.

All studies involving animal or human subjects must abide by the rules of the appropriate Internal Review Board and the tenets of the recently revised Helsinki protocol. Hence, the manuscripts must include the name of the ethics committee that approved the study and the committee's reference number if appropriate.

6. Results

The Results section should concisely describe the findings of the study including, if appropriate, results of statistical analysis which must be presented either in the text or as tables and figures. It should follow a logical sequence. However, the description of results should not simply repeat the data that appear in tables and figures and, likewise, the same data should not be displayed in both tables and figures. Any chemical equations, structural formulas or mathematical equations should be placed between successive lines of text. The authors do not discuss the results or draw any conclusions in this section.

7. Discussion

The Discussion section should focus on the interpretation and the significance of the findings against the background of existing knowledge. The discussion should not repeat information in the results. The authors will clearly identify any aspects that are novel. In addition, there is the relation between the results and other work in the area.

8. Conclusions

The Conclusions section should state clearly the main summaries and provide an explanation of the importance and relevance of the study reported. The author will also describe some indication of the direction future research should take.

9. Acknowledgements

The Acknowledgements section should be any brief notes of thanks to the following:

- *Funding sources*
- *A person who provided purely technical help or writing assistance*
- *A department chair who provided only general support*
- *Sources of material (e.g. novel drugs) not available commercially*

Thanks to anonymous reviewers are not allowed. If you do not have anyone to acknowledge, please write "Not applicable" in this section.

10. References

The Vancouver system of referencing should be used in the manuscripts. References should be cited numerically in the order they appear in the text. The authors should identify references in text, tables, and legends by Arabic numerals in parentheses or as superscripts. Please give names of all authors and editors. The references should be numbered and listed in order of appearance in the text. The names of all authors are cited when there are six or fewer. When there are seven or more, only the first three followed by "et al." should be given. The names of journals should be abbreviated in the style used in Index Medicus (see examples below). Reference to unpublished data and personal communications should not appear in the list but should be cited in the text only (e.g. A Smith, unpubl. Data, 2000).

- *Journal article*

1. Sibai BM. Magnesium sulfate is the ideal anticonvulsant in preeclampsia – eclampsia. Am J Obstet Gynecol 1990; 162: 1141 – 5.

- *Books*

2. Remington JS, Swartz MN. Current Topics in Infectious Diseases, Vol 21. Boston: Blackwell Science Publication, 2001.

- *Chapter in a book*

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11. Tables

The tables should be self-contained and complement, but without duplication, information contained in the text. They should be numbered consecutively in Arabic numerals (Table 1, Table 2, etc.). Each table should be presented on a separate page with a comprehensive but concise legend above the table. The tables should be double-spaced and vertical lines should not be used to separate the columns. The column headings should be brief, with units of measurement in parentheses. All abbreviations should be defined in footnotes. The tables and their legends and footnotes should be understandable without reference to the text. The authors should ensure that the data in the tables are consistent with those cited in the relevant places in the text, totals add up correctly, and percentages have been calculated correctly.

12. Figure Legends

The legends should be self-explanatory and typed on a separate page titled "Figure Legends". They should incorporate definitions of any symbols used and all abbreviations and units of measurement should be explained so that the figures and their legends are understandable without reference to the text.

If the tables or figures have been published before, the authors must obtain written permission to reproduce the materials in both print and electronic formats from the copyright owner and submit them with the manuscripts. These also follow for quotes, illustrations, and other materials taken from previously published works not in the public domain. The original resources should be cited in the figure captions or table footnotes.

13. Figures

All illustrations (line drawings and photographs) are classified as figures. The figures should be numbered consecutively in Arabic numerals (Figure 1, Figure 2, etc.). They are submitted electronically along with the manuscripts. These figures should be referred to specifically in the text of the papers but should not be embedded within the text. The following information must be stated to each microscopic image: staining method, magnification (especially for electron micrograph), and numerical aperture of the objective lens. The authors are encouraged to use digital images (at least 300 d.p.i.) in .jpg or .tif formats. The use of three-dimensional histograms is strongly discouraged when the addition of these histograms gives no extra information.

14. Components

14.1. Letters to the Editor

The Letter to the Editor manuscripts consist of the following order:

- *Title Page*
- *Main Text*
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- *Figure Legend (if needed)*
- *Figure (if needed)*

14.2. Original Articles

The Original Article manuscripts consist of the following order:

- *Title Page*
- *Structured Abstract*
- *Introduction*
- *Materials and Methods*
- *Results*
- *Discussion*
- *Conclusions*
- *Acknowledgements*
- *References*
- *Table (s)*
- *Figure Legend (s)*
- *Figure (s)*

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The Review Article manuscripts consist of the following order:

- *Title Page*
- *Unstructured Abstract*
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- *References*
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- *Figure Legend (s)*
- *Figure (s)*

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The Case Report manuscripts consist of the following order:

- *Title Page*
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- *Introduction*

- *Case Description*
- *Discussion*
- *Conclusions*
- *Acknowledgements*
- *References*
- *Table (s)*
- *Figure Legend (s)*
- *Figure (s)*

14.5. Case Illustrations

The Case Illustration manuscripts consist of the following order:

- *Title Page*
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- *Multiple Choice Questions (MCQs)*
- *Take-Home Messages (Learning Points)*
- *Acknowledgements*
- *References*
- *Correct Answers to MCQs*
- *Table (s)*
- *Figure Legend (s)*
- *Figure (s)*

14.6. Technical Notes

The Technical Note manuscripts consist of the following order:

- *Title Page*
- *Introduction*
- *Main text*
- *Conclusions*
- *Acknowledgements*
- *References*
- *Table (s)*
- *Figure Legend (s)*
- *Figure (s)*

Proofreading

The authors of the accepted manuscripts will receive proofs and are responsible for proofreading and checking the entire article, including tables, figures, and references. These

authors should correct only typesetting errors at this stage and may be charged for extensive alterations. Page proofs must be returned within 48 hours to avoid delays in publication.

Revised Manuscripts

In many cases, the authors will be invited to make revisions to their manuscripts. The revised manuscripts must generally be received by the Editorial Board within 3 months of the date on the decision letter or they will be considered a new submission. An extension can sometimes be negotiated with the Editorial Board.

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Since AAP is also a peer-reviewed journal, the submitted manuscripts will be reviewed by the members of the Editorial Board or the expert reviewers. The decision on these manuscripts is processed very fast without any delay and in shortest possible time. The processing period is 1 – 2 weeks. These decisions of the reviewers are unbiased and the decision (reject, invite revision, and accept) letter coming from the Editorial Board is always conveyed to the authors.

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Step 5: Proceed to fill up the Submission Form online and follow the directions given therein.

Step 6: Upload your manuscript file (s).

Step 7: Re-check the content of your manuscript (s) and the uploaded file (s) more carefully prior to the submission. If you have submitted your manuscript file (s) incorrectly, you must contact Editor-in-Chief of Asian Archives of Pathology immediately. The Editor-in-Chief can clear the incorrect attempt and allow you another submission.

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Assistant Professor Dr Chetana Ruangpratheep
The Editorial Office of Asian Archives of Pathology
Department of Pathology, Floor 6, Her Royal Highness Princess Bejaratana Building
Phramongkutklao College of Medicine
317 Rajavithi Road, Rajadevi, Bangkok 10400 Thailand
Telephone: +66 (0) 90 132 2047
Fax: +66 (0) 2 354 7791
Email: editor@asianarchpath.com

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MD, FRCPath (Thailand), MSc, PhD
Editor-in-Chief of Asian Archives of Pathology

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